STUDIES ON THE INTERACTION OF NEMATODES AND FUNGI ON CERTAIN FOREST TREES

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INTRODUCTION
&
REVIEW OF LITRATURE

CHAPTER I

INTRODUCTION & REVIEW OF LITERATURE

Forests are considered as national wealth as sources of timber, fuel, fodder and minor forest products and they help in conserving soil and water, moderating climate etc.

According to FAO report (1963) the area under forest on earth is 3779 million hectares,while in India it is 75 million hectares, constituting 23% of the total forest area. This area is decreasing due to mass deforestation partly due to urbanisation resulting in low produce.

The productivity of forest is also getting low due to inherent slow growth of the species, diseases and insect pests.

The ever increasing requirements for various raw products is putting more and more pressure to foresters to have the maximum yield of forest products per hectare in the shortest possible time. In order to meet the demand, the whole concept of forest management is changing and is coming on line with agricultural crop management. The foresters are now concerned in improving planting stock, and are adopting improved cultural techniques and latests technology in controlling pests and diseases.

There are large number of diseases of forest trees caused by fungi such as root rot, wilt, etc., which are responsible for reducing the economic value of the trees. (Bakshi, 1972).

In nursery, damping-off is the most serious of all the nursery diseases and has a wide distribution. The symptoms of damping off in conifers are caused by species of *Pythium, Rhizoctonia, Phytophthora* and *Fusarium* (Roth & Riker, 1943; Tint, 1945). In India *R. solani* is the important cause of damping-off in nurseries while *Pythium* and *Fusarium* are less commonly recorded (Reddy, 1969)

Eucalyptus seedlings suffer from damping off caused by *Fusarium oxysporum, Macrophomina Phaseolina* and *Rhizoctonia solani* (Taha *et al* 1987). *Corticium salmonicolor* cause cankers on *Eucalyptus grandis* (Subramaniam & Ramaswamy, 1987).

Mortality of seedlings of *Eucalyptus* spp. was caused by *Curvularia lunata, Fusarium semitectum, Helminthosporium* and *Myrothecium roridum* (Saxena, 1985).

Post-emergence damping off of *Eucalyptus* was caused by *Fusarium solani* (Michail etal. 1986). This fungus was found seed borne in *Eucalyptus*. Taha *et al.* (1987) observed that *Eucalyptus* damping-off was caused by several fungi such as *Fusarium oxysporum, Macrophomina phaseolina* and *Rhizoctonia solani. M. phaseolina* was the most virulent, followed by *R. Solani*. Perrin (1986) also confirmed that *Pythium* and *Fusarium* spp. and *Rhizoctonia solani* are the main pathogens of damping off and root rot in a forest nursery.

The wilt caused by *Verticillium* sp. was very commonly found attacking large number of trees. Sisso trees are susceptible to wilt disease due to *Fusarium vasinfectum* (Bakshi 1976). These trees in advanced age are attacked by *Ganoderma lucidum* and *Polyporus gilvus.* The former causes root disease and die back, while the latter produces stem canker in trees.

Randhawa *et al.* (1986) reported that *R. stolonifer* was the most common fungus associated with rotten seeds and abnormal seedlings of *Cassia fistula,* although *Aspergillus flavus, A. niger* and *Penicillium* sp. were also recorded. Seeds soaked in conc. H_2 So_4 for 5 min. and immersed in *R.* stolonifer *suspension produced a higher proportion of rotted seeds and abnormal seedlings.*

Seedlings are also subjected to collar rot due to *Rhizoctonia solani* (Perrin, 1986).

The root rots are caused by *Armillaria mellea, Ganoderma lucidum, Fomes annosus* and *Polyporus schneinitzii.* Some of these fungi are also responsible of causing rot of heart and sap wood (Bakshi, 1976).

All these diseases take a heavy toll of nursery seedlings every year and thus reduce the productivity.

Acacia trees are susceptible to *Fomes pappianus,* which causes the wood to become brittle. Heart and root rot fungi (Phaeolus schweinitzii, Polyporus sulphureus, Pleurotus ostreatus) as well as *Armillaria mellea* and *Ganoderma* spp. were considered the major factor in deterioration of *Acacia Koa* (Robert, 1979).

Fusarium, Verticillium, Phoma, Phomopsis, Pestalotia, Botryodiplodia, Curvularia species are also important pathogens of various trees. (Carneiro, 1986).

Damage to trees is also caused by different species of *Botrytis* on forest trees, seedlings and seeds (Mittal *et al.* 1987). It is also a cause of threat to reforestation. *Sparassis radicata* causes root rot of forest trees in general (Martin & Gilbertson, 1976).

Nectria ditissima is responsible of causing canker diseases of hardwood trees (Capretti & Mugnai 1987), while *Fomes annosus* is a serious problem in several tree seedlings and causing butt--rot in conifers (Rishbeth, J. 1951). *Stereum hirsutum, S. gausapatum* and *S. frustulatum* are responsible of causing heart rot of oak.

Subramaniam & Ramaswamy (1987) while studying the histopathological changes in *Eucalyptus* due to fungus invasion, found that *Corticium salmonicolor* caused cankers, which under favourable conditions completely girdled the stem. At low levels of infection, fungal spread was obstructed by the formation of tyloses which plugged the vessels. A deviation from normal cambial behavior was also observed.

Nayar, & Ramanujam, (1985) recorded *Ailanthus malabarica* as new host for *Pestalotia algeriensis* and *Knema attenuata* for *Cephaleuros virescens*.

Lunoquist (1987) pointed out that *Diplodia pinea* infected mainly *Pinus radiata* and *P. patula; Armillaria mellea* two species of *Eucalyptus* and eight species of pine principally *P. roxburghii; Helicobasidium compactum* pines and *Pseudophaeolus bandonii* seven species of *Eucalyptus* and three species of *Pinus*.

Katumoto & Harada (1987) gave illustrated account of plant parasitic ascomycetes such as *Sphaerulina myriadea* and *Gnomonia setacea* on oak *(Quercus dentata)*, *Mamianiella coryli* var. *coryli* on *Corylus sieboldiana* and *Hypospilina oharana* (a new comb.) on elm (*Ulmus davidiana* var. *japonica*.

Cytospora eucalypticola, *Paecilomyces variotii* and *Phialophora bubakii* were the most commonly isolated organisms from *Eucalyptus bancroftii* and *E. macrorhyncha* after 3 and 6 months of mechanical injury to the sapwood (Wilkes 1987).

Weste & Marks (1987) observed destruction of whole natural plant communities in Australia by *Phytophthora cinnamoni*. *Phytophthora cinnamoni* and *Pythium* spp. were detected in thinned *Eucalyptus* plots (Gerrettson *et al*, 1979). *Phytophthora cryptogea*, *P. drechsleri*, *P. megasperma*, *P. cactorum* were responsible of causing root rot in several forest tree nurseries (Hansen *et al*, 1979).

Ann & Ko (1988) reported *Ganoderma lucidum* and *Kretzschmaria clavus* causing root rot of *Macadamia integrifolia* trees. In some cases decay extended to the main trunk and eventually caused death of the tree. Infected tissues of 80% of diseased trees turned soft due to fungus *G. lucidum*. The remaining declining trees were infected by *K. clavus* which formed black, lines on infected tissues. In pathogenicity tests, 40 and 80 percent of macadamia branches were killed by *G. lucidum* and 20 and 60 percent were killed by *K. clavus* after 3 and 6 months of inoculation respectively.

Filip (1979) observed that mortality of *Pseudotsuga menziesii* was caused by either *Armillaria mellea* or *Phellinus weirii*. *Armillaria* was responsible of causing root-rot in softwood plantations (Singh, 1975). Singh (1989) observed root-rot caused by *Armillaria* spp., *Heterobasidium annosum*, *Phellinus weirii* and *Inonotus tomentosus* on several forest trees. *Armillaria*, *Cephalosporium*, *Cladosporium*, *Cylindrocarpon* and *Sporothrix* spp., *Diplodia mutila*, *Hypoxylon mediterraneum*, *Phoma cavae* and *Phomopsis quercina* were isolated from declining oaks (Ragazzi *et al*. 1989).

Carneiro (1986), while studying the mycoflora associated with seeds of forest trees, found *Fusarium*, *Verticillium*, *Phoma*, *Phomopsis*, *Botryodiplodia*, *Pestalotia*, *Rhizoctonia* and *Curvularia* as predominant fungi. Frequency of these fungi was more when seeds were stored at room temperature than those stored at low temperature.

Motta (1986) found *Seiridium cardinale* on seeds of some cupressaceae and *Fusarium moniliforme (Gibberella fujikuroi)* and its var. *subglutinans* on seeds of *Pinus pinaster*.

Perrin (1987) while studying the diseases caused by *Rhizoctonia solani* found that acidophilic soils were less favourable to infection than calcareous soils.

Singh & Mittal (1989) studied the influence of seed-borne fungi on growth and nutrient composition of conifer seedlings. Untreated and chilled seeds of *Pinus strobus* and *Picea glauca* were inoculated with *Cladosporium cladosporioides*, *Fusarium sporotrichioides*, *Mucor hiemalis* or *Trichothecium roseum* and sown in sterilized soil, unsterilized soil or a peat vermiculite perlite mix. Fungal inoculation resulted in changes in the concentration of various macro and micro nutrients (N, P, K, Mg, Na, Ca, Mn, Fe, & Zn). There was reduction in the height, growth and biomass of the resulting seedlings. No relation was found between changes in nutrient level and reductions in height, growth or biomass and no consistent effect was caused by chilling of the seeds. It was suggested that the fungi caused some kind of interference in the normal physiology of germination of seed and seedling development including absorption and/or translocation of nutrients resulting in depletion of some ions and accumulation of others and that these physiological disturbances affected the height, growth and biomass.

Another group of organisms which are soil inhabitants i.e. plant nematodes are also no less important than fungi. No systematic approach has been done to assess the losses due to nematodes but there is awareness that they are one of the causes of decline in the productivity of the trees.

Plant nematodes have only recently been recognised as problems to trees specially in nurseries. Large number of nematodes have been reported from both aerial parts and rhizosphere of trees. *Aphelenchoides fragariae* in the cuttings of *Fiscus* spp (De Maeseneer, 1964), *A. besseyi* on *Fiscus elastica* (Marlatt, 1966), *Aphelenchoides* sp. on *Areca* palm (Thirumalachar,

1946) *Rhadinaphelenchus cocophilus* on coconut palms (Singh, 1970) *Elaeis guineensis* (Oostenbrink, 1963) are some of the important nematodes reported from aerial parts of the forest trees *Aphelenchoides* sp. caused bud rot of *Areca* palms in India in which infected trees became thin crowned; they gradually shed their leaves & finally died (Thirumalachar, 1946).

The infection of *R. cocophilus* which causes red-ring disease of coconut palm (Fenwick and Maharaj 1963) is manifested into yellowing and browning of leaves followed by withering and death of the growing point and finally of trees. Internally it forms red discoloration in the cortical tissues in the form of a ring.

The nematodes found associated with trees in the rhizosphere can be grouped into two categories :

1) nematodes feeding on mycorrhizal symbionts.
2) nematodes directly feeding on roots Riffle (1967) reported that *Aphelenchoides* sp. associated with mycorrhizae of trees decreased the growth of aerial and substrate mycelium of symbiotic fungi like *Suillus*

graulatus and *Mycelium radicis atrovirens* in laboratorty. Similarly *Aphelenchus avenae* has been found feeding on mycorrhizal fungi and inhibited the formation of association on red pine (Sutherland and Fortin, 1968).

The nematodes directly feedings on roots are mainly those associated with nurseries. As early as 1932, *Pratylenchus* sp. was reported associated with *Picea* sp. Since then large number of nematodes such as *Helicotylenchus* sp., *Hoplolaimus* sp., *H. galeatus, Longidorus maximus, Meloidodera floridensis, Meloidogyne* sp., *Pratylenchus* sp., *P. penetrans, Rotylenchus robustus, Trichodorus* sp, *Tylenchorhynchus* sp, *T. claytoni, T. ewingi, Xiphinema americanum, X. bakeri* and *X. diversicaudatum* have been found associated with the rhizosphere of various forest trees, mostly belonging to temperate areas (Ruehle 1973). Details of trees wtih which these nematodes have been reported associated are given in Table -I.

Henry (1953) although reported root damage in some species of *Pinus* but he could not account the damage due to nematodes because of low population.

However, Weischer (1956) observed brown root lesions on conifers growing in nematode-infested areas. Some doubts were raised about the stunting and yellowing of *Picea, Pinus* and *Larix* in nurseries due to nematode, *Rotylenchus robustus. Pratylenchus penetrans, Tylenchorhynchus* sp. and *Ditylenchus* sp. were commonly found associated with forest tree seedlings (Nolte and Dieter, 1957). Later the association of above mentioned species of nematodes causing damage to the roots of *Pinus montana mughus, Picea pungens, P. abies* and *P. sitchensis* was also confirmed (Ruehle). Oostenbrink (1958) also reported *R. robustus* damaging the roots of *Pinus* sp.

Hopper (1958) observed stunted and unthrifty growth of pine seedlings due to *Meloidodera floridensis, Tylenchorhynchus claytoni* and *Tylenchorhynchus* spp. Although *M. floridensis* was found in the roots of *Pinus clausa, P. nigra, P. palustris,* and *P. taeda* but severe damage was caused only to *P. clausa.*

Chitwood and Esser (1957) proved that *M. floridensis* is pathogenic to *P. elliottii*. When inoculated with high population of larvae of *M. floridensis*, the root development was reduced and the mature female nematodes were visible protruding through the epidermis of roots. The females caused compression and collapse of the cortical cells (Ruehle 1962).

Tylenchorhynchus claytoni and *T. ewingi* were commonly associated with chlorotic and dying of *P. elliottii* having roots short and stubby in clusters. Ruehle (1969) observed damage of roots of *P. elliottii*, *P. palustris* and *P. taeda* under controlled conditions. The affected seedlings were severely stunted and lacked fine feeder roots with few or none mycorrhizae. High populations of nematodes particularly of *Xiphinema americanum* and *Criconemoides xenoplax* were responsible for declining and unthrifty growth of *Picea pungens*, *P. glauca* var. *densata* (Epstein and Griffin 1962).

Goodey (1965) found the association of *Rotylenchus robustus*, *Trichodorus pachydermus* and *Tylenchorhynchus* spp. with *Larix leptolepsis*, *Picea sitchensis*, *Pinus contorta*, *P. nigra* var. *poiretiana* and *Pseudotsuga menziesii* in forest nursery soil but *R. robustus* was reported pathogenic to *Picea sitchensis*. *R. robustus* was also found pathogenic to *Picea abies* in nursery (Hijink, 1969).

In Japan, *Cryptomeria japonica*, *Chamaecyparis obtusa*, *Pinus* sp. and *Acacia* sp. were parasitised by *Meloidogyne incognita* *Tylenchorhynchus claytoni* and *Pratylenchus* sp. (Hashimoto, 1962). The galls caused by root-knot nematode on *Acacia* were larger than those found on conifers. *Tylenchorhynchus claytoni* caused browning and withering of feeder roots by feeding of nematodes. The damage to seedlings was more severe in old nurseries where the population build up was high. *Pratylenchus penetrans* also caused considerable damage to coniferous seedling. It was however, widely distributed in nurseries throughout Japan.

In Brazil, *Meloidogyne arenaria* caused heavy galling on seedlings raised from seeds imported from Japan. The infested seedlings were stunted and developed galls with 75% mortality (Lordello and Kanazawa, 1967).

Seedlings of *Anthocephalus chinensis* were severely stunted with galls on roots in Philippine due to *Meloidogyne incognita* (Postrado and Glori 1968). *Xiphinema bakeri* was consistently associated with *Pseudotsuga menziesii* resulting in roughened, thickened, and browned roots (corky roots) (Bloomberg 1968) throughout Canada. This nematode fed on tips of seedling roots.

Another aspect of nematode problem in forest trees is in forest plantations. They have nematode problems different to those of nurseries. In natural forests, the nematodes develop an ecological balance with trees and little damage is observed. The nematode numbers are low to cause damage. In such areas intensive cultivation encourages high population of plant nematodes. This has often resulted in serious "re plant" problem. Stunting of *Pinus taeda, P. elliottii* has been found to be due to *Hoplolaimus galeatus Meloidodera floridensis, Helicotylenchus dihystera* and *Xiphinema americanum* (Ruehle and Sasser 1962). Other nematodes found associated with these trees, were, *Belonolaimus* sp., *Criconemoides* sp. *Dolichodorus* sp., *Hemicriconemoides* sp., *Hemicycliophora* sp., *Longidorus* sp. and *Pratylenchus zeae. X. americanum* has also been found commonly associated with *Populus deltoides, Ulmus* spp., *Pinus* spp. *Fraxinus pennsylvanica* and *Celtis occidentalis* (Malek, 1968). Unthrifty growth of trees was found to be due to *X. americanum, Tylenchorhynchus acutus* and *Paratylenchus* sp. Churchill and Ruehle (1971) reported that *Hoplolaimus galeatus* was pathogenic to *Platanus occidentalis* in greenhouse.

In natural woodlands, *Meloidodera floridensis* was found infecting roots of *Pinus rigida* and *P. echinata* (Hutchinson and Reed 1959) and *Pseudotsuga menziesii* (Hirschmann and Riggs 1969). *Heterodera betula* infected roots of birch (Riggs and Hamblen, 1967). Most of the nematodes generally cause die back problems. In addition to above, several other nematodes have also been found associated with die back or slow decline problem of trees. (Table II).

Lal & Khan (1987) while making a survey of nematodes associated with forest trees found that the population mainly consisted of *Coslenchus diversus, C. erectus* and *Filenchus brevis* in the rhizosphere of *Cupressus torulosa, Acacia auriculiformis* and *Juglans regia*.

Helicotylenchulus spp. and *Criconemella* spp. have been widely reported associated with forest trees in nurseries and reserved natural forests (Lal & Khan, 1988).

Basirolaimus indicus was found associated with *Cassia fistula, Shorea robusta* and *Tectona grandis*; *Rotylenchulus* *reniformis* with *C. fistula*, *S. robusta* and *Tylenchorhynchus mashhoodi* with *Stzygium comnis*; *Xiphinema insigne* with *T. grandis* (Ray & Das, 1985). Macgowan (1988) reported *Helicotylenchus pseudorobustus*, *Radopholus similis*, *Xiphinema vulgare*, *Xenocriconemella macrodora*, *Meloidogyne* species and *Trichodorus* sp. on bamboo *(Bambusa glaucescens)*; *H. pseudorobustus*, *Hoplolaimus galeatus*, *Xiphinema americanum*, *Xiphinema* sp., *Criconemoides xenoplax*, *X. macrodora*, *R. similis*, *Trichodorus porosus* on bamboo *(B. tuldoides)*. *Hemicriconemoides wessoni*, *Hoplolaimus tylenchiformis*, *Meloidodera floridensis*, *Trichodorus christiei*, *X. americanum*, *Criconemoides* sp. and *X. macrodora* on feathery bamboo *(B. vulgaris)*. *Xiphinema luci* and *Tylenchulus palustris* on seaside oxeye *(Borrichia arborescens)* and *H. wessoni*, *Heterodra* sp., *H. galeatus*, *Meloidogyne* sp., *T. porosus* and *X. americanum* on male bamboo *(Dendrocalamus strictus)*. *Acacia arabica* and *Azadirachta indica* were reported as new hosts of *Meloidogyne incognita* (Dahiya *et al.* 1988).

Mujib (1986) observed that the length and dry weight of shoot and roots of *Leucaena leucocephala* decreased as the inoculum density of *M. incognita* increased. Reduction in growth was associated with stunting ,yellowing, browning and defoliation. Severe galling on roots was also observed. However, on *Areca catechu* no root galling or egg mass formation was noted for 3 to 9 months after inoculation with *M. arenaria*, *M. hapla*, *M. incognita* races 1 and 3, or *M. javanica*. The significant reduction in growth of palms was reported when plants were infected with *M. incognita* race 1 and not with other species. (Mcsorley & Dunn 1989). *Bursaphelenchus Xylophilus* (pine wood nematodes) is found responsible of causing cambium destruction in conifers (Myers, 1986).

Ronald (1986) obtained a correlation of rate of mortality of 2-4 year old conifers with the number of pine wood nematodes *Bursaphelenchus xylophilus*. Percentage of conifer mortality was, however, more for spring inoculations when cambial activity was greater than for late summer and fall inoculations. Seedlings of *Pinus clausa* infected with large numbers of *Hoplolaimus galeatus* showed decrease in length and weight of roots (Ruehle, 1972).

Although *Dubosica myoporodies* was reported a new host of *M.*

incognita, *Pratylenchus coffeae*, *Helicotylenchus imperialis* and *Tylenchorhynhus digitatus* (Rajan *et al.* 1987) but seedlings grown in pots showed stunted growth in the presence of root-knot nematode only. Die back symptoms, however, were observed under field conditions only when all the four nematode genera were present in the rhizosphere. Pradhan & Dash (1987) studied the distribution and population dynamics of soil nematodes in tropical forest ecosystem. Seventeen species of nematode were identified of which *Rotylenchus* sp. was the dominat plant parasitic form and *Acrobeloides* sp. was the dominat microbivore species. Nematodes were distributed in clusters resulting in a so called pocket effect. Of the total nematodes 88.4% occurred in the top 10cm of soil during the peak period of density and the microbivores were more frequent in the top 5 cm of soil. Total nematode density ranged from $15.1 \times 10^4/m^2$ (May) to $66.1 \times 10^4/m^2$ (Nov). The mean monthly nematode biomass was 18.86 ± 8.36 mg dry wt./m^2 temperature, soil organic carbon and soil total nitrogen apparently played an important role in regulating the nematode population.

Lal and Khan (1988) identified 82 spp. of nematodes associated with forest trees. Quantitatiye estimation of nematodes revealed higher population densities of "tylenchids" (mostly feeders on roots of higher plants) in forest nurseries, while higher population densities of "Aphelenchids" and "mononchids" (mostly predators on other nematodes) were encountered in natural forests. Frequency of occurrence of some major plant-parasitic genera encountered showed the highest frequency of *Xiphinema* spp. (62.7%) followed by *Helicotylenchus* spp. (46%) and *Criconemella* spp. (42%).

Seedlings of trees are raised in soil which harbour large number of fungi (both pathogenic and saprophytic) and nematodes (parasitic and saprozoic). Most of the studies have been carried out in identifying the problems of tree growth either caused by fungi or nematodes but in nature it seldom happens. The pathogenic fungi and parasitic nematodes interact each other and also interact with other soil biota. The total outcome in a disease is interaction of sum total of interaction in amongst all the soil microorganisms in addition to other factors like environment, host etc. Practically no information is available on the interaction of nematodes and fungi in nurseries on growth of forest tree seedlings but some doubts have been raised, that the microorganisms interact. Sutherland and Dunn (1970) isolated *Cylindrocarpon radicicola* from diseased roots of *Pseudotsuga menziesii*,where the population of *Xiphinema bakeri* was high.

De Maeseneer (1964) also isolated *C. radicicola* together with *Fusarium oxysporum* from nematode infested conifer seedlings. It is likely that there might be interaction of nematode and fungi in the decline of conifer seedlings. Dropkin & Foudin(1979) obtained *Bursaphelenchus lignicolus* together with *Diplogaster* spp. Cultures of *B. lignicolus* were established on cultures of the fungus *Botrytis cinerea*.

Fungus-nematode relations of corky root disease of Douglas fir were reported by Bloomberg & Sutherland (1971). In diseased roots the primary cortex persisted and enlarged, but secondary vascular tissues, periderm, pericycle and endodermis were reduced. *Cylindrocarpon destructans* was the fungus most frequently isolated from cortical cells. *Fusarium oxysporum* or *Mycelium radicis atrovirens* were abundant. Populations of the plant-parasitic nematodes,particularly *Xiphinema bakeri*,on roots and in adhering soil were highest 2 months after germination and during the summer correlating positively with the percent of tap root affected and negatively with numbers of lateral roots.

The role of *Aphelenchus avenae* in the appearance of Fusarium disease of *Pinus sylvestris* seedlings was studied by Kulinich (1985) *P. sylvestris* was inoculated with *F. oxysporum* var. *mycophylum* or *F. graminearum (Gibberella zeae)* with or without addition of *A. avenae* . Number of seedlings obtained (taken as 100% in normal soil controls) was 93-96% in pots with nematodes only, 30-53% with *Fusarium* and *Aphelenchus* and 16-22% with the fungi alone.

Rohde *et al* (1986) pointed out that *Bursaphelenchus xylophilus* may not be the prime factor in causing pine wilt disease because of the presence of blue stain fungus. It could be caused by both the organisms. The wilt disease complex on conifers was found associated with *Bursaphelenchus xylophilus*, *Aphelenchoides* sp., bark beetles, blue staining fungi and the root rotting fungus, *Inonotus tomentosus*. (Bergdahl *et al*, 1986).

A direct correlation between levels of *Radopholus similis* population present in soil and growth and intensity of root lesions in *Areca catechu* seedlings were recorded by Sundraraju & Koshy (1987). The nematode in combinations with *Cylindrocarpon obtusisporum* caused more damage in inoculations where nematode was introduced three weeks prior to fungus. The fungus alone did not cause any appreciable damage. Nematode multiplication was inhibited in the prsence of fungus.

Considerable work on the interaction of nematodes and fungi on damage of plants has been done on agricultural crops. The literature has been reviewed by Pitcher (1965) and Powell, (1971).

In such interaction there has been involvement of a variety of nematodes with wilt and root-rot causing fungi (Powell, 1971) on several agricultural crops. But, unfortunately there is paucity of information on this aspect in forest tree seedlings which suffer a lot in nurseries.

With increase in urbanisation throughout the world, there is an emerging awareness of the value of urban forests for improving environment. Trees located near human habitation provide shade and shelter against sun and wind; yield timber and fruit in orchards; serve as a screen for privacy and camouflaging, provide an aesthetic beauty to the landscape; play a role of a natural filter purifying the air from dust and in effectively changing the concentration of harmful gases.

Amongst the different urban forest trees in Aligarh *Albizzia lebbck*, and *Bauhinia purpurea* are widely grown on road side and also in yards around houses. Besides this, wood is used for agricultural implements and the bark for tanning.

Two root and buttrot fungi viz. *Polyporus zonalis* and *Hypoxylon ustulatum* frequently occur together and cause damage to these plant. They cause decay of roots resulting in windthrow of affected trees. Besides there may be many more fungi and nematodes in the rhizosphere which might be influencing the germination of seeds and growth of seedlings of plant. But some how no work has been done on the aspect of the interaction between the organisims.

It is, therefore, intended to make a systematic study of the rhizosphere mycoflora and nematodes of *Albizzia lebbek* and to work out if there is any interaction between the two groups of organisms in decline in the growth of seedlings of the plant.

The detailed plan of work is as follows :

1- Isolation and identification of fungi and nematodes from rhizosphere and roots of plants of diferent ages of *Acacia arabica*, *Albizzia lebbek*, *Delbergia sisso*, *Cassia fistula* and *Bauhinia* and determination of population fluctuation of nematodes in different season.

2- Effect of certain fungi: Known as antagonistic to pathogen and pathogenic fungi on germination of seeds and growth of seedlings when incorporated into soil separately. This would aim to select the fungus which is pathogenic and also the one which enhances the germination of seeds.

3- Effect of root-knot nematode and other predominant nematodes on germination of seeds and growth of seedlings.

4- Effect of culture filtrate of the fungi screened [in (2)] above, on germination of seeds and on mortality of nematodes and hatching of root-knot nematode larvae.

5- Effect of inoculating the seedlings of different ages with root-knot nematode larvae and other nematodes on growth of plants and biomass (and penetration of larvae and development of nematodes in case of root knot nematode).

6- Effect of inoculation of seedlings with root knot nematode larvae/other nematodes and the pathogenic fungus on growth of plants and biomass (and on penetration of larvae and development of females in case of root knot nematode).

7- Effect of inoculation of seedlings with root knot nematode larvae/other nematodes and the saprophytic fungus on growth of plants and biomass (and on penetration of larvae and development of females in case of root knot nematodes).

8- Effect of inoculation of seedlings with root knot nematode larvae, the saprophytic fungus and the pathogenic fungus on growth of plants and biomass (and on penetration of larvae and development of females in case of rootknot nematode).

MATERIAL

&

METHODS

CHAPTER II

"MATERIALS & METHODS"

2.1 RAISING OF SEEDLINGS:

To raise the forest tree seedlings, seeds presoaked in water for 48 hours and treated with captan, will be sown in sterilized soil.

Seedlings thus raised will be used for various studies.

2.2 ISOLATION OF FUNGI AND NEMATODES FROM RHIZOSPHERE:

2.2.1(i) Fungi:

The seedlings of plants in different ages will be removed from soil and brought to the laboratory in sterilized containers under aseptic conditions. Soil adhering to the roots will be removed and crushed gently without damaging the roots. The root pieces thus obtained will be placed in 100 ml of sterilized distilled water and shaken to obtain maximum possible of soil. This will be used as stock solution. Different dilutions upto 1:1000 will be made from stock solution. Then 1 ml of 1:1000 dilution will be placed in sterilized petridishes containing 10 ml of molten, cooled peptone dextrose agar * or potato dextrose agar **. The petriplates will be rotated to obtain equal distribution of soil suspension in the medium.

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*Peptone-dextrose agar

KH_2PO_4	1.0 g
Peptone	5.0 g
Dextrose	10.0 g
Agar	20.0 g
Distilled water	1000 ml
Rose bengal	1:30,000 sol
Streptomycin	30 ug/ml

**Potato-dextrose agar (PDA)

200 g peeled potatoes, which will be cut into small chips, boiled in 500 ml of water, extract mixed with 20 g agar and 20 g dextrose boiled already in 500 ml of water.

For each treatment 20 petriplates will be poured. The plates will be incubated at 20^{o}C for one week. Fungi appearing will be examined and identified.

For studying rhizoplane mycoflora "serial root washing technique" (Harley and Waid, 1955) will be followed. Roots will be washed several times in sterilized distilled water and cut into small pieces of 5mm length and transferred into sterilized petriplates with 5 root pieces in each petriplates containing 10 ml of sterilized melted, cooled peptone dextrose agar medium. Pertiplates will be incubated at 20-28^{o}C. Fungi will be examined and identified.

The frequency of fungi in both cases will be calculated as follows:

$$\frac{\text{Number of plates containing a fungus}}{\text{Total plates poured}} \times 100$$

The fungi will be isolated in pure culture either by making single spore or hyphal tip isolation (Riker & Riker, 1936). The fungi so purified will be maintained on sterilized potato dextrose agar (PDA) slants.

2.2.2(ii) <u>Nematode</u>

(a) <u>from roots:</u>

The roots indicating infection will be cut into small pieces measuring 0.5mm in length. The weighed amount of pieces will be placed in warring blender for a few seconds. The macerated roots will be poured over coarse sieve lined with tissue paper and placed over a funnel filled with water having the neck with a rubber tube and stop cork. After every 24 hours the nematode suspensions will be taken out and examined under the stereoscopic microscope. Nematodes of different genera will be counted.

(b) <u>from soil:</u>

Nematodes from soil will be isolated by using cobb's sieving and decanting technique. The soil sample will be properly spread and mixed and a sub-sample of 250 g will be used for isolation. The soil will be then mixed with water and passed through sieves of various meshes. The supernatant collected after passing through 300 mesh sieve will be placed over a coarse sieve lined with tissue paper as for isolation of nematodes from roots. The nematodes recovered after 24 hrs. will be examined and counted.

2.3 PREPARATION OF FUNGAL INOCULUM:

The inoculum of fungus would be raised on Czapek's sucrose nitrate solution (Levine and Schoenlein, 1930) medium of the following constitution:

$NaNo_3$	2.0 g
K_2HPo_4	1.0 g
$MgSo_4.7H_2o$	0.5 g
Kcl	0.5 g
$FeSo_4.H_2o$	0.018 g
Sucrose	30.0 g
Water (Distilled)	1000 ml

This medium would be sterilized and then will be transferred to 250 ml Erlenmeyer flasks with 25 ml medium in each flask.

After 15 days of inoculation the incubated mycelial mat will be filtered through Whatman filter paper No.1 The mycelium will be washed with distilled water to remove the traces of medium. It will be gently pressed between the folds of sterile blotting paper to remove the excess amount of water.

The fungus (10 g) mat will be put in blender to macerate in 100 ml of sterilized water. The macerated mycelium will be used for inoculation. Thus each 10 ml of this suspension will contain 1g of fungus.

2.4 INOCULATION WITH FUNGUS:

For inoculation, a portion of the soil will be removed from around the roots of plants and the seedlings will be inoculated by pouring required amount of suspension so as to make 1g, 5g and 10g fungus per seedling.

2.5 PREPARATION OF INOCULUM OF ROOT-KNOT NEMATODE:

Eggmasses will be collected from roots of plants infected with root-knot nematode *(Meloidogyne incognita)* from single eggmass culture beds. The eggmasses so picked will be treated with 0.01% chlorox to separate the eggs. The eggs will be transferred to distilled water contained in petridishes for hatching into larvae. The larvae so hatched will be used for inoculation. Seedlings will be inoculated with 10, 100, 1000 and 10,000 larvae/Kg. soil to determine the extent of losses and the minimum threshold.

In subsequent studies the level of nematode to inoculation to be kept will be above the minimum threshold.

For others, the nematodes will be picked and collected in distilled water to make suspension of required numbers (10, 100, 1000 & 10,000 per Kg. soil).

2.6 INOCULATION WITH NEMATODES:

The suspension of nematode having required number of juveniles or nematodes will be poured near the root zone by making four holes at equidistance around the stem.

2.7 EFFECT OF FUNGI AND NEMATODES ON GERMINATION OF SEEDS AND GROWTH OF SEEDLINGS:

Sterilized soil (1 Kg.) contained in 15 cm earthenware pots will be inoculated with different fungi and nematodes/larvae separately and together.

After inoculation seeds (10) will be sown in each pot. Germinating seeds will be counted after every 24 hrs. and finally after 15 days, all the seedlings will be uprooted and growth will be determined by measuring length and weight of root/shoot. There will be ten pots for each treatment in one replicate. Seeds will be sown in uninoculated soil for control.

Seedlings of plants in different ages (2,4,6 & 8 week old seedlings,) will be inoculated with different fungi (important pathogens) and nematodes separately and in different combination.

2.8 EFFECT OF CULTURE FILTRATE OF DIFFERENT FUNGI ON SEED GERMINATION:

The fungi isolated in the rhizosphere will be grown on (Czepek's medium for 15 days. The fungal mat will be removed by filtration and the culture filtrate will be collected.

This culture filtrate will arbitrarily be named as "Standard". It will be diluted to 10^{-1}, 10^{-2}, 10^{-3} with sterile distilled water. Seeds will be presoaked in different concentration of the culture filtrate and will be sown in sterilized soil. Germination of seeds and seedling growth will be determined by measuring length and weight of root/shoot.

Presoaking of seeds with water will serve as control. Each pot will have 10 seeds and there will be 10 pots for each treatment. Each treatment will be replicated thrice.

2.9 <u>EFFECT OF NEMATODES & FUNGI ON GROWTH OF PLANTS:</u>

Seedlings of different ages (1,2,5,10 week old) raised in sterilized soil will be inoculated with nematodes (10,100,1000 & 10,000)/Kg. and fungi (1,5 & 10 g)/kg. soil separately to determining the pathogenicity. Growth of plants will be determined after 60 days of inoculation. There will be 10 plants for each treatment with three replicates.

In studies dealing with interaction of namatode and fungi on growth of plants, inoculation will be done as under:

1- Uninoculated (Control)

2- Nematode

3- Fungus 1

4- Fungus 2

5- Nematode + Fungus 1

6- Nematode + Fungus 2

7- Fungus 1 + Fungus 2

8- Nematode + Fungus 1 + Fungus 2

There will be 5 plants for each treatment and each treatment will be replicated thrice.

2.10 <u>EFFECT OF CULTURE FILTRATE:</u>

(a) <u>On hatching of Larvae</u>

Culture filtrate of different fungi will be obtained by growing them in (Czepek's medium)

After growing the fungus for 15 days, the contents of the flasks will be filtered through Whatman filter paper. The filtrate will be termed as "standard" solution and will be diluted to 10^{-1}, 10^{-2}, & 10^{-3} with sterilized distilled water.

The culture filtrate (10 ml) will be poured in 5cm petridishes to which 20 freshly collected eggmasses will be added. The filtrate of different fungi will be tested separately and in combination. The petridishes will be incubated at 20°C.

After 2,4,6,8 days the number of larvae hatched out will be counted. Hatching in distilled water will serve as control.

(b) <u>On Mortality of Nematodes:</u>

Nematode suspension with known number of nematodes will be incorporated in the culture filtrate contained in 5 cm petridishes of different concentrations. Number of nematodes showing no mobility will be counted. These nematodes will then be transfer to distilled water to determine if they regain mobility. Those not regaining mobility will be considered as dead and the percentage of mortality will be determined.

A similar study will be made with root-knot nematode larvae hatched in distilled water. There will be five replicates to each treatment. Each treatment in a replicate will have 10 petridish.

2.11 **PLANT GROWTH DETERMINATION:**

Plants will be uprooted after 60 days of inoculation. Root system will be thoroughly washed with running water. For measuring the length and weight the plants will be cut just above the base of root emergence zone.

The excess water of plant will be removed by putting them between two folds of blotting sheets before weighing for fresh weight.

For measuring dry weight, the plants will be first kept in an oven at 60^{o}C to dry for 2-3 days. After cooling dry weight of roots and shoots will be determined separately.

ROOT-KNOT ESTIMATION:

Intensity of root-knot will be categorized as follows :

Category	No. of galls
1	0-50
2	51-100
3	101-150
4	151-200
5	201-250

For determining the penetration of larvae/nematodes the finer roots will be stained with cotton blue in lactophenal. The stain will be heated till simmering. The roots will then be transferred to lactophenol for clearance and examined under the microscope. The number of larvae penetrated will be counted.

2.12 NEMATODE POPULATION ESTIMATION:

For extraction of nematodes, soil from each treatment will be mixed thoroughly and a sub-sample of 200 g soil will be processed thoroughly through "Cobb's seiving and decanting method" followed by Baermann funnel technique (Southey 1979).

The suspension will be collected in a beaker and make it upto 100 ml. For proper distribution of nematodes the suspension will be stirred and aerated with pipette and 2ml suspensioon will be transferred to a counting dish (Southey, 1970). The number of nematode will be counted. Five countings will be made. Mean will be taken and this population of nematodes/Kg. of soil will be calculated.

The population of nematodes will be determined in different seasons i.e. January, March, June, July, August, October and December.

TABLE I. Plant-parasitic nematodes frequently associated with diseased forest tree seedlings in nurseries

Nematode species	Host genera or species	Location	Reference
Helicotylenchus sp.	*Picea pungens*	U.S.A. (Mich.)	Knierim (1963)
Hoplolaimus sp.	*Picea pungens*	U.S.A. (Mich.)	Knierim (1963)
H. galeatus	*Pinus*	U.S.A. (Fla)	Steiner (1949)
Longidorus maximus	*Abies.Alnus,Acer,Pinus, Larix.Carpinus,Robinia, Quercus,Corylus,Picea,Thuja*	Germany	Sturhan (1963)
Meloidodera floridensis	*Pinus*	U.S.A. (Fla)	Hopper (1958)
Meloidogyne sp.	*Acacia*	Japan	Hashimoto (1962)
	Pinus	U.S.A. (Fla)	Donaldson (1967)
		Japan	Hashimoto (1962)
	Cornus, Catalpa	U.S.A. (Ga)	Johnson *et al.* (1970)
	Anthocephalus	Philippines	Postrado and Glori (1968)
Pratylenchus sp.	*Cryptomeria, Chamaecyparis, Pinus, Picea*	Japan	Yamaguchi (1932)
	Picea pungens	U.S.A. (Mich.)	Knierim (1963)
P. penetrans	*Picea, Taxus, Juniperus, Cedrus, Thuja, Abies, Pinus, Pseudotsuga*	Belgium	De Maeseneer (1964a)
	Picea	Germany	Ruhm (1959)
	Cryptomeria	Japan	Mamiya (1969)
	Picea, Pinus	Canada	Sutherland (1967)
			Brande (1965)
Rotylenchus robustus	*Picea, Acer*	England	Goodey (1965)
	Juniperus, Thuja, Taxus, Cedrus, Picea, Pseudotsuga	Belgium	De Maeseneer (1964a)
	Pinus	Belgium	De Maeseneer and van den
Trichodorus sp.	*Pinus*	U.S.A. (Ala)	Hopper and Padgett (1960)
	Picea	England	Goodey (1965)
	Acer	Belgium	De Maeseneer and van den Brande (1965)
Tylenchorhynchus sp.	*Picea*	England	Goodey (1965)
	Taxus	U.S.A. (Mich.)	Knierim (1963)

Nematode species	Host genera or species	Location	Reference
T. claytoni	*Picea, Pinus*	U.S.A. (W. Va)	Sutherland and Adams (1964)
	Pinus	U.S.A. (Ala)	Hopper (1958)
	Cryptomeria, Chamae-cyparis	Japan	Hashimoto (1962)
T. ewingi	*Pinus*	U.S.A. (La)	Hopper (1959)
Xiphinema americanum	*Abies*	U.S.A. (Wis.)	Griffin and Darline (1964)
X. bakeri	*Pseudotsuga*	Canada	Sutherland and Dunn (1968)
X. dirersicaudatum	*Abies, Larix, Pinus, Alnus, Carpinus, Acer, Robinia*	Germany	Sturhan (1963)

TABLE II. Plant-parasitic nematodes associated with decline problems of trees in natural woodlands

Nematode species	Host genera orspecies	Location	Reference
Criconema sp.	*Pinus monticola*	Idaho	Nickle (1960)
	Fraxinum americanum	New York	Ross (1966)
Criconemoides sp.	*F. americanum*	New York	Ross (1966)
Helicotylenchus sp.	*Acer saccharum*	New York	Hibben (1964)
	Fraxinus americanum	New York	Ross (1966)
	Pinus ponderosa, P. edulis, Juniperus monosperma, J. deppeana, J. scopulorum	New York	Riffle (1968)
H. platyurus	*Acer saccharum*	Wisconsin	Riffle and Kuntz (1966)
Hemicycliophora sp.	*A. saccharum*	New York	Hibben (1964)
		Wisconsin	Riffle and Kuntz (1966)
H. vidua	*Pinus echinata*	Alabama, Georgia, N. Carolina, S. Carolina, Virginia	Ruehle (1962b)
Heterodera sp.	*P. monticola*	Idaho	Nickle (1960)
Hoplolaimus	*P. echinata*	Georgia,	Ruehle (1962b)
galeatus		N. Carolina, Virginia	
Longidorus sp.	*Fraxinus americanum*	New York	Ross (1966)
Meloidodera floridensis	*Pinus echinata*	New Jersey	Hutchinson and Reed (1959)
Meloidogyne ovalis	*Acer saccharum*	Wisconsin	Riffle and Kuntz (1967)
Pratylenchus sp.	*Fraxinus americanum*	New York	Ross (1966)
Trichodorus sp.	*F. americanum*	New York	Ross (1966)
T. californicus	*Acer saccharum*	Wisconsin	Riffle and Kuntz (1966)

Nematode species	Host genera or species	Location	Reference
T. elegans	*Pinus monticola*	Idaho	Nickle (1960)
Tylenchorhynchus sp.	*P. monticola*	Idaho	Nickle (1960)
	Fraxinus americanum	New York	Ross (1966)
Xiphinema sp.	*Acer saccharum*	New York	Hibben (1964)
	Fraxinus americanum	New York	Ross (1966)
X. americanum	*Acer saccharum*	Massachusetts	Di Sanzo and Rohde (1969)
		Wisconsin	Riffle and Kuntz (1966)
	Pinus echinata	Georgia, Alabama, N. Carolina, S. Carolina, Virginia	Ruehle (1962b)
	P. ponderosa, P. edulis, Juniperus monosperma, J. deppeana, J. scopulorum	New York	Riffle (1968)

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